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Prevalence of Escherichia coli serogroups and human virulence factors in faeces of urban Canada geese (Branta canadensis)

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This was the first study to exhaustively characterize the prevalence of *Escherichia coli* sero-groups in any wildlife species. Faecal samples from Canada geese (*Branta canadensis*) were collected over a single year in Fort Collins, Colorado, USA. The overall prevalence for *E. coli* ranged from 2% during the coldest time of the year to 94% during the warmest months of the year. During the time of year when nonmigratory geese dominated the local goose population (March–July) the prevalence of enterotoxogenic (ETEC) forms of *E. coli* was 13.0%. The prevalence of enterohemorrhagic (EHEC) forms was 6.0%, while prevalence for enteroinvasive (EIEC) and enteroagglomerative (EAEC) forms was 4.6 and 1.3%, respectively, during the same period. We also examined all samples positive for *E. coli* for genes coding for virulence factors, including: SLT-I, SLT-II, *eae, hly-A*, K1, LT, STa, STb, CNF1, and CNF2. Three isolates were positive for human virulence factors, representing a 2% prevalence for faeces containing potential human toxins. Genes for STa were isolated from ETEC strains O-8 and O-167, while the gene for K1 was isolated from an O-8 (ETEC) serogroup. These data will prove useful in focusing attention on the risks that increasing populations of urban Canada geese pose to public health.

Introduction

Increasingly, large numbers of Canada geese (*Branta canadensis*) occur in urban parks, recreation areas, and corporate and residential lawns. While few of the public ever come in direct contact with these geese, they frequently come into contact with goose faeces and faecally contaminated water and lawns. As a consequence, the public and health officials have questioned what human health risks are associated with this faecal contamination (Conover and Chasko 1985; Cooper and Keefe 1997).

In this study we described the prevalence of *Escherichia coli* in Canada goose faeces collected over an 11-month period in Fort Collins, Colorado. Although *E. coli* is part of a normal gut flora of vertebrates, virulent forms do exist (Hussong *et al.* 1979). It is the potential presence of these virulent forms of *E. coli*, as well as other zoonoses (e.g., Converse *et al.* 2001; Roscoe *et al.* 2001), that are of interest to health officials in assessing whether the faecal contamination of parks represents a human health risk factor.

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E. coli that cause human diarrheal illness are classified into groups based upon their virulence properties, mechanisms of pathogenicity, clinical symptoms, and distinct O:H sero-types (Nataro and Kaper 1998). Although surveys for the prevalence of *E. coli* in avian species have been published (Brittingham 1988; Harris 1991; Aguirre 1992; Feare *et al.* 1999; Alderisio and DeLuca 1999), the specific strains of *E. coli* generally have not been characterized as to their serotype or virulence factors.

We undertook this study with the following objectives: (i) to quantify the magnitude of Canada goose faecal load from urban landscapes as an index of human exposure to goose faeces, and as an index of goose population density for a given study site; (ii) to determine whether there was a relationship between faecal load and prevalence of *E. coli*; (iii) to determine whether there were any seasonal patterns for the prevalence of *E. coli*; (iv) to characterize *E. coli* as to their serogroup, and; (v) to characterize *E. coli* for the presence of genes responsible for the production of various human virulence factors.

Methods

Study site description and sampling schedule

Four sites within Fort Collins, Colorado, USA, were selected based on preliminary surveys as being representative of locations with high and low goose activity. Faecal accumulation on fixed sample transects was used as an index of this activity (Mason and Clark 1995, 1996). Each site was characterized as a corporate or residential lawn of approximately 2 acres composed of Kentucky Blue Grass and included the presence of open water. Within each site, three transects (0.6 × 30.5 m each) were established by marking the lawn with spray paint. Once a week for three consecutive weeks the transects were raked to clear away accumulated faeces. Three transects were used to minimize bias estimates on the degree to which geese used areas of turf within the site. On the fourth week all of the faeces occurring within the transect were collected, stored in a zip-lock freezer bag, and transported to the laboratory for analysis. In the laboratory, aggregate samples from each transect were dried to constant weight in ovens (60°C) ~ 48 h, and then weighed. The resulting dry weight served as an index of usage of the site by geese and incorporates information about the amount of time geese spent on a site as well as population size (Mason and Clark 1995, 1996). Higher accumulated dry weights can also be used to infer higher probabilities of humans coming in contact with goose faeces.

During the same period when aggregate faecal samples were collected, we also collected fresh Canada goose faecal samples adjacent to the transect. Goose faeces are readily distinguishable from other waterfowl, bird and mammal faeces. That the faeces were produced by Canada geese was assured because random visits to the sites indicated that Canada goose were the only goose species to visit the sites. Fresh faeces were identified as being firm but moist relative to concurrently available faecal material. No attempt was made to collect liquidy faeces indicative of diarrhea. Care was taken to collect the fresh faeces over a large area adjacent to the transects where the aggregate faecal samples were collected. This procedure minimized the possibility that we over sampled faeces derived from individual geese. We made no effort to obtain cloacal cultures because the principal risk to the public, if any, is not with contact from geese. Rather, the potential health risk to the public is with contact with faeces. The issue of whether variously aged faeces contain viable human pathogens is the subject of another study. This study focused on fresh faecal material only. Fresh faecal material was placed into sterile whirl-packs using aseptic techniques and transported to the laboratory for culture within one to three hours of collection.

Bioassays

Standard culture procedures were used for culture and isolation of *E. coli* (Nataro and Kaper 1998). Faeces were smashed and stirred within the sample bag and a small sub-sample was extracted with an innoculating loop and directly smeared onto MacConkey agar plates and incubated at 37°C for 24 h. Positive and negative controls were analyzed concurrently. Well isolated dark pink colonies indicative of strong lactose fermenters were subcultured onto blood agar plates and inoculated at 37°C for 24 h. Well defined isolated colonies that were light tan to grey were suspected *E. coli* and subjected to three biochemical confirmation tests: KOH, oxidase, and indole. For each test, suitable positive and negative controls were included. Colonies yielding a putative identification for *E. coli* from the biochemical and blood agar tests were subcultured onto MacConkey with sorbitol agar plates which is a selective media for *E. coli* O157:H7 (Isenberg 1992). These cultures were incubated at 37°C for another 24 h. In addition, AP120E strips were used to provide putative identifications of all isolates.

Sixty-two isolates tested positive for *E. coli* and were collected in March, and May through July 1999. These isolates were stored in 3 µl of tryptic soy broth and 3 µl of glycerol at –70°C for subsequent further characterization. Isolates were recultured by inoculation onto nutrient agar slants and submitted to the Penn State Gastro-enteric Wiley Laboratory for serogrouping and toxin assays. Details of the procedures and quality assurance of antisera are posted on the Wiley Lab's web site (http://ecoli.cas.psu.edu). Isolates were screened in a presumptive assay using monovalent rabbit antisera reactive with 181 O sero-groups, with positive reactions confirmed by microtitration assays. Antisera were checked and assured for quality in August 2001. Faecal samples were then classified as being positive and containing serogroups belonging to one of the following sero-groups: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC), or World Health Organizations (WHO) standard strains of *E. coli*.

O serogroup screening as described above for each isolate consisted of adding 20 µl of each of 181 O antisera into V-bottom 96-well culture plates. To the wells, 180 µl of diluted antigen were added and the plates were incubated at 50°C for 24 h. Agglutination was evaluated with the aid of a micotiter plate viewer, with negative reactions appearing as white buttons at the bottom of the plate and positive reactions as being cloudy to clear. Confirmation for each positively reacting isolate consisted of placing 10 µl of antiserum into the first well of a row on a V-bottom 96-well plate to which was added 190 µl of phenol saline (6 g NaCl, 6 ml phenol brought to 1l with distilled water). To the remaining wells in the row were added 100 µl of phenol saline. The first well was mixed and 100 µl were transferred to the second well in the row. The process was repeated, thus serially diluting the antiserum for each well (n = 7) with the exception that the last well in the row was not diluted. The final titers in wells 1–7 ranged from 1:80 to 1:51. Plates were incubated overnight in a humidity chamber at 50°C. Reactions were scored as described above.

The presence for cytonecrotizing factor 1 and 2 (CNF1, CNF2), heat-labile enterotoxin (LT), heat-stable enterotoxin a and b (STa, STb), Shiga-like toxin I and II (ST-I, ST-II), *eae*, K1, and *hyl-A* genes was determined by a polymerase chain reaction test (PCR) for all positive *E. coli* isolates under contract to the Wiley *E. coli* Reference Center, Pennsylvania State University, State College, Pennsylvania. This method uses repeated cycles of oligonucleotide-directed DNA synthesis to perform in vitro replication of targeted nucleic acid sequence (http://ecoli.cas-.psu.edu. Ehrlich and Greenberg 1993). The oligonucleotide primers selected for amplification of the various toxin genes were appropriate for Canada-geese. Strains for positive controls were as follows: LT and STb (strain 80.2575, sero-typed O157:K88:H13), STa (strain B41M,

sero-typed O101:NM), K1 (strain U9–41, sero-typed O2:K1:H4). Negative control strains used were: *E. coli* K12 and C600, and water.

Results

We collected 397 Canada goose faecal samples from the four study sites from October 1998 to August 1999. *E. coli* were present in 147 of the samples (37.0%). There was no relationship between the faecal load at each site and the overall prevalence of *E. coli* (Fig. 1, $r^2 = 0$, $P = 1.0$). However, there was a pronounced positive correlation between ambient temperature and

Table 1. Mean temperature compared to the prevalence of *Escherichia coli* throughout the study in Fort Collins, Colorado

Mean temperature (°C)[a]	*Mean (g)*[b]	*± SE*	*No. of samples*[c]	*Prevalence (%)*	*Month*
10.72	70.5	20.73	13	46	October
3.17	41.73	9.44	20	30	November
8.45	93.29	44.63	32	9	December
–0.51	49.65	1.13	40	8	January
3.68	63.85	13.75	52	2	February
8.88	54.04	3.46	48	25	March
8.05	17.45	34.9	36	19	April
9.32	6.29	46.61	52	39	May
16.18	42.31	8.82	25	80	June
22.41	23.62	28.43	26	77	July
22.53	95.33	46.77	52	94	August

[a] The mean air temperature in Fort Collins.
[b] The mean dry weight of faeces collected per transect (18.3 m^2), $n = 12$. Means were calculated by averaging over the four sampling locations, with each location consisting of three transects.
[c] The total number of fresh faecal samples taken from the four sites.

Table 2. Prevalence of *E. coli* O-serogroups classified by associated pathology grouping isolated from Canada goose faeces in Fort Collins, CO as a function of time

Associated Pathology	*March (N = 48)*		*May (N = 52)*		*June (N = 25)*		*July (N = 26)*		*Total (N = 151)*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Standard WHO Strain	4	6.3	7	13.5	4	16.0	10	36.5	25	16.6
ETEC	4	6.3	7	13.5	5	20.0	3	11.5	19	13.0
EIEC	3	6.3	0	0	2	8.0	2	7.7	7	4.6
EHEC	0	0	4	7.7	3	12.0	2	7.7	9	6.0
EAEC	0	0	0	0	2	8.0	0	0	2	1.3

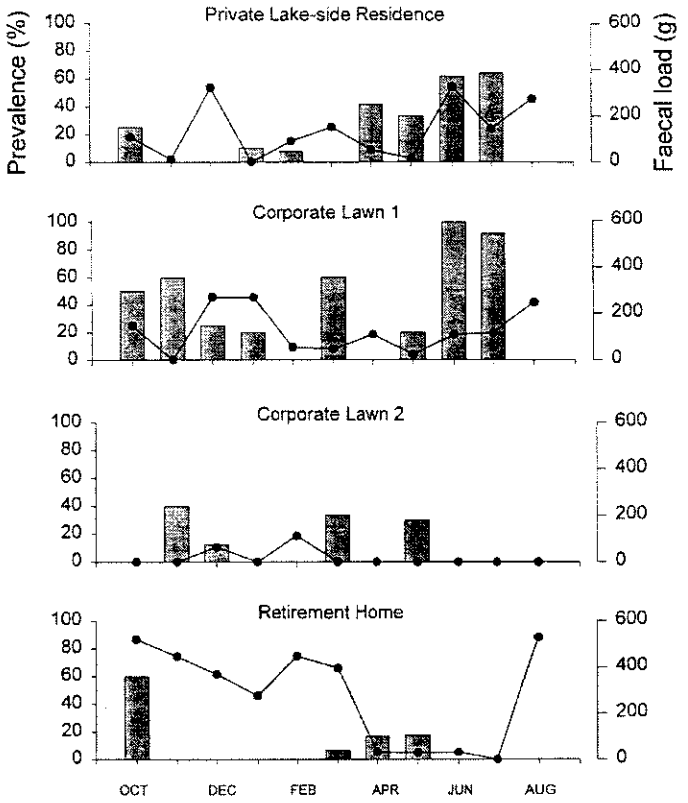

Fig. 1. Profiles of prevalence for *E. coli* (shaded bars) derived from faecal samples and the total dry weight of goose faeces (point and lines) collected during a 7-day period for each of the four study sites as function of time of year.

prevalence for *E. coli* in faeces (r^2 = 0.823, df 1,9, F = 41.983, P < 0.001). Overall, prevalence for *E. coli* ranged from 2% during the colder months to 94% during the warmest months (Table 1). However, local conditions were also important for how geese used sites and for the prevalence patterns for *E. coli* (Fig. 1).

We characterized the faecal samples in greater detail between March and July. Of the 151 faecal samples collected during this period, 62 (41.1%) were positive for *E. coli* (Table 2). Generally, we isolated only one dominant strain of *E. coli* per faecal sample. Standard O strains (World Health Organization definition) were found in 16.4% of the faecal samples. No one WHO standard strain was found throughout the samples (Table 3). *E. coli* strains consistent with those associated with human illness were isolated from 24.5% of the faecal samples. Serogroups associated with enteropathogenicity were most commonly isolated from faeces (13.0%), with O-8 being the most prevalent ETEC strain (Table 3). Two ETEC isolates tested positive for the presence of genes for heat stable toxin a (STa). These were found in single isolates belonging to the O8 and O167 sero-groups. One ETEC isolate belonging to the O8 serogroup showed the presence of gene sequences for K1, which is related to meningitis and avian respiratory disorders. These isolates were not confined to a single site. Rather they were isolated from faeces at discrete locations within Fort Collins: a corporate lawn, a private

Table 3. Identity and frequency of *E. coli* O-serogroups classified by associated pathology grouping isolated from Canada goose faeces in Fort Collins, CO

Standard WHO Strain		*ETEC*		*EHEC*		*EIEC*		*EAEC*	
Sero-group	*n*	*Sero-group*	*n*	*Sero-group*	*n*	*Sero-group*	*n*	*Sero-group*	*n*
9	1	6	1	5	1	28	2	3	1
13	2	8	16	18	2	152	1	86	1
36	1	15	1	x25	1	159	4		
54	2	167	1	118	1				
56	1			91	3				
65	1			146	1				
74	1								
79	1								
83	1								
88	1								
102	2								
105	2								
106	1								
113	1								
116	1								
123	1								
132	1								
141	1								
16/89/162*	3								

* Weak reaction positive identification not possible.

lake-side residence, and a retirement home. No O157:H7 isolations were identified, although 6.0% of the samples were classified as EHEC strains. All isolates were negative for the following toxin genes: CNF1, CNF2, *eae, hly* A, LT, SLT1, SLT2, and STb. Approximately 4.6% of the strains belonged to sero-groups associated with EIEC forms of *E. coli*, while 1.3% belonged to strains associated with EAEC.

Discussion

The overall prevalence pattern described for *E. coli* in this study is similar to the patterns found in other studies. Feare *et al.* (1999) found *E. coli* in approximately 50% of cloacal and faecal samples derived from Canada geese in London parks. Hussong *et al.* (1979) reported enterotoxigenic bacteria from waterfowl without specification. Converse *et al.* (2001) failed to find *E. coli* O157:H7 in a survey of Canada geese in the eastern United States. However, they did not assay for other hemorrhagic forms of *E. coli*. There remains the possibility that O157:H7 might be detected in goose faeces during other times of the year, such as during the migration and overwintering periods. These migratory populations frequently travel between urban sites and rural settings where they might come into contact with stock pastures and faeces, and hence be exposed to environmental sources of pathogenic *E. coli* (Kudva *et al.* 1998). We do not

believe this to be the case in this study. Geese do move from the urban to rural environment during the fall and winter. However, the rural fields visited around Fort Collins tend to be harvested grains and have not been subjected to fertilization with manure at that time of year. Also, being an arid environment, free-ranging dairy herds are not common.

This is the first study to exhaustively characterize *E. coli* sero-groups derived from faeces of any wildlife species. While sero-grouping does not provide information about the presence or absence of specific human virulence factors, it has been an historically reliable correlate indicating association with specific disease states (Sears and Kaper 1996; Nataro and Kaper 1998; Chattopadhyay *et al.* 2001). Moreover, we identified human virulence factors, specifically two isolates for STa and one for K1 for a combined prevalence of 2% of faecal samples. The low prevalence of human virulence factors and the absence of clusters of human diarrhea cases in Fort Collins may suggest that Canada goose faeces does not pose a significant human health risk. However, we argue that such a dismissal may be unwarranted. Because geese and their faeces are distributed across urban landscapes it may be more difficult to identify disease clusters should they occur. Should clinical cases be reported, data characterizing *E. coli* sero-groups from urban goose populations may prove useful in identifying potential risk factor sources. For example, a higher than expected frequency of O-8 sero-groups identified in clinical evaluations might occur. By itself this information would certainly not be conclusive. However, it could direct officials to investigate the possibility of geese as a source of infection.

The mere presence of large numbers of geese on lawns, and by association, large quantities of faeces, have raised the public's concern about the safety of parks, sports fields, and golf courses (Feare *et al.* 1999; Fallacara *et al.* 2001). Yet little is known about what real health risks these geese might pose to the public. Ideally inferences of health risk of goose faeces to humans should be based upon the probability of encounter rate with virulent forms of *E. coli*. However, we often lack the detailed information about the prevalence for virulence factors that is necessary to make park and goose management decisions (Feare *et al.* 1999). Information about seasonal goose behavior, faecal distribution patterns, environmental conditions, prevalence of virulent strains of bacteria, and recreational use patterns of parks by humans are needed to estimate the probabilities for which humans may encounter virulent strains of bacteria.

Based on the observations obtained in this study we illustrate a scenario for a possible encounter rate of an individual taking a 1-mile walk in a park. That individual would take approximately 3,500 strides, and with an average imprint of 0.03 m^2 per stride, the individual would come in contact with approximately 106 m^2 of turf. The hiker's encounter rate with faeces will vary, depending upon location and time of year. Using dry faecal weights per transect (Table 1) and the average dry faecal weight of an individual piece of faeces (1.34 g $\pm$ 0.10 SEM, $n = 50$), the hiker might encounter from none to four pieces of faeces per meter during a walk. For example, based on values in Table 1, there was an average of 95 g of faeces per transect in August. Dividing by 1.34 g per individual piece of faeces yields 71 faeces per transect. Each transect is 18.3 m^2, thus, the density of faeces would be approximately 4 m^{-1}. Multiplying this value by the total area of contact by the hiker's shoe (106 m^2) yields an estimated faecal encounter rate of 424 pieces per walk. At a 2% prevalence the hiker is likely to come into contact with eight pieces of faeces containing virulent strains of *E. coli*. The likelihood of infection of course would depend upon additional factors, such as the hiker's natural resistance to challenge, and the behavior and hygiene practices of the hiker after they remove their shoes.

We emphasize that this scenario is an illustration of how an estimate of risk can be assessed for public lawns. It is not intended to provide a unitary definitive value for the calculation of risk to human health that Canada geese pose. However, as more details about seasonal variations in

prevalence and local goose abundance are acquired, this information could be used in the development of cogent wildlife management and public health plans. If the level of human health risk is perceived to be too high, public health officials could post alerts in areas of goose use indicating to the public that sanitary precautions should be taken. These precautions would include recommendations that contact with turf and faeces should be minimized, that shoes should be removed before entering homes, and hands should be washed thoroughly with antibacterial soaps. Alternatively, wildlife managers could act to reduce local goose populations such that the estimated faecal encounter rate is reduced to an acceptably low level.

As mentioned, several factors will affect the prevalence of virulent strains of *E. coli* found in goose faeces, with survivability of the bacterial strains being the most obvious factor. Warmer temperatures will promote growth and survivorship better than colder temperatures, as evidenced in Table 1. Humidity and precipitation (natural and irrigation) will affect dessication rates of faeces. Moister faeces provide for a more favorable environment for bacterial growth and survivorship. For example, *E. coli* O157:H7 were able to grow and proliferate for up to 2 days in fresh chicken manure at 20°C, with increases of 1–2 log units in cfu. Thereafter increasing free ammonia concentrations in the faeces lowered survivorship. Survivorship was also lowered by drying the manure (Himathongkham and Riemann 1999). While the longevity of *E. coli* in goose faeces held under environmentally fluctuating conditions is unknown, studies on *E. coli* O157:H7 survivorship in ovine and bovine manure yielded longevities from 47 to 630 days (Kudva *et al.* 1998).

The overall prevalence for all strains of *E. coli* in Fort Collins did not correlate to faecal density, and by implication extant goose numbers. Rather, it was positively related to prevailing warmer seasonal temperatures, being higher in the spring and summer and lower during the fall and winter. Besides these obvious environmental correlates to overall prevalence the behavior of geese may also have contributed to the prevalence patterns. During the fall and winter, despite there being large populations of migratory and nonmigratory geese present, the daily movement patterns of the birds largely are concentrated onto dry upland harvested grain fields outside of town and on turf within the town. These birds are not likely to come in contact with habitats contaminated with mammalian sources of *E. coli*. In contrast, during the spring and summer the goose population consists of nonmigratory birds. These birds do not move far from their nests during breeding. Moreover, the habitat consists of small water impoundments and littoral zones that easily become fouled. When the birds do range from their nest area they are more likely to visit outlying agricultural areas that are surface-treated with manure from local feedlots and dairies. Several studies have shown that environmental contamination from pathogenic and nonpathogenic strains of *E. coli* occurs with this practice of manure spreading (Kudva *et al.* 1998; Gagliardi and Karns 2000; Ogden *et al.* 2001). Thus, it is arguable that resident birds may be (1) exposed to greater levels of environmental *E. coli*, (2) that these birds will tend to concentrate any potential bacterial contamination over a smaller area because of their decreased mobility, and (3) that the survivorship of *E. coli* in faeces is longer because of optimal growth conditions. Together these events may translate to cause for concern for the health and well being of the public utilizing public lawns and parks. Should the concern warrant action is a matter of public debate relative to the costs and benefits that wildlife provide. Actions may consist simply of public health advisories, harassment of geese to reduce numbers, lethal removal of geese, or treatment of the turf with agents that lessen growth and survivorship of potentially pathogenic bacteria. For example, carbonate anion has been used to kill *E. coli* in dairy cattle manure (Arthurs *et al.* 2001; Jarvis *et al.* 2001), and may provide a means to manage lawn contamination from *E. coli* in goose faeces in public parks.

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